ARE ‘APPARENT’ SEX REVERSED CHINOOK SALMON A SYMPTOM OF GENOTOXICITY?

Bernie P May

Final Selection Panel Review

Proposal Title

#0318: ARE ‘APPARENT’ SEX REVERSED CHINOOK SALMON A SYMPTOM OF GENOTOXICITY?

Funding:

Fund
Amount: **$143,735**

The final Selection Panel concurred with its initial findings on this proposal and recommended funding the proposal at the full amount requested.

Public Comments

No public comments were received for this proposal.

Initial Selection Panel Review

Proposal Title

#0318: ARE ‘APPARENT’ SEX REVERSED CHINOOK SALMON A SYMPTOM OF GENOTOXICITY?

Funding:

Fund in part
Amount: **$143,735**

Initial Selection Panel (Primary) Review

Topic Areas

- Life Cycle Models And Population Biology Of Key Species
- Environmental Influences On Key Species And Ecosystems
- Salmonid−related Projects

Please describe the relevance and strategic importance of this proposal in the context of this PSP. How does the proposal address the topic areas identified above? What are the broader CALFED Goals this proposal may meet that are not accounted for in these specific topic areas?

This proposal would provide information that can help elucidate an area of salmon biology concerning sex reversal. This proposal also touches on the area of the role of contaminants in this "apparent" sex reversal. If successful, the results of this work would provide potentially valid information for management of Central Valley hatcheries. However, it is not clear if the techniques employed can be used on a real time basis for hatchery management. I don't see the relevance of this proposal to management of natural salmon production.

The budgets of proposals submitted in response to this PSP are larger, on average, than those submitted to CALFED in previous years. The Science Program is committed to getting as much science per dollar as is reasonably possible. With this commitment in mind, can the

proposed budget be streamlined? If so, please recommend and clearly justify a new budget total in the space provided.

The budget appears reasonable and necessary.

Evaluation Summary And Rating.

Provide a brief explanation of your summary rating and any additional comments you feel are pertinent.

This proposal does not appear to address a critical unknown in the management of the Bay−Delta system. While this is a missing piece of information in what has been a series of studies by the authors, it does not seem well suited to funding under the Calfed Science PSP. Calfed is relying on natural production to improve the Bay−Delta resources and has not been interested in funding improvements to Central Valley hatchery management.

Selection Panel (Discussion) Review

fund this amount: $**143,735**
note:
fund in part

This is one step in a series of fish genetics work by this group looking at apparent female salmon who have XY rather than XX. Eventually they want to look at toxicity as a cause, but here they propose to investigate chromosomal changes responsible for XY females.

The Panel felt that this is a polished proposal with a strong team.

The proposers suggest the applicability of this research would potentially be to remove these XY/apparent females from hatchery brood stock. However, CALFED is not relying on hatchery production to meet its goals, so this is not a realistic goal. This work could influence production estimates, particularly in winter−run JPE (Juvenile Production

Estimates), where assumptions are currently based on 50:50 sex ratios. These estimates of carrying capacity of habitat may change based on this work. The authors are not proposing to evaluate what causes the mutation.

The Panel was divided as to whether the budget is reasonable. For example, May is supposed to be getting salary for cytogenetics, but Phillips is slated to do the work. Additionally, $17,000 for cytogenetics supplies seems high. The Panel was also divided as to whether this is a high priority item. The resolution was that this research would complement proposal #111. Thus, this proposal is recommended for funding if combined with #111 to ensure collaboration between the two proposals.

Panel Ranking: Fund with modifications.

Collaboration Panel Review

Proposal Title

#0318: ARE ‘APPARENT’ SEX REVERSED CHINOOK SALMON A SYMPTOM OF GENOTOXICITY?

Final Panel Rating
adequate

Collaboration Panel (Primary) Review

Collaboration:

Will the results of the collaborative effort be greater than the sum of its parts? Is it clear why the subprojects are part of a larger collaborative proposal rather than several independent smaller ones?

adequate
Not clear – all participants are genetics (not multi-disciplinary) but apparently collaborative.

Interdependence And Integration:

Does the proposal have an example that clearly articulates the conceptual model of each subproject and how they link together as a whole? Are the boundaries of the study plans focused and cohesive, yet well delineated? Is there a plan for potential differences in the stages of subproject completion times? Are there clear plans for analyses and interpretations which seek to identify and quantify relationships among the data collected in various subprojects rather than separate analyses for each subproject?

adequate
Conceptual model described. Studies focused and delineated. No plan for differences in completion times. No description of analyses.

Project Management:

Is it clear who will be performing management tasks and administration of the project? Are

there resources set aside for project management and time given for investigators to collaborate? Is there a process for making decisions during the course of the project? Are there acknowledgments of potential barriers to collaboration and explanations of how team members will overcome barriers particular to their institutions?

adequate
Project manager identified, but resoures do not seem to be set aside, nor time for collaboration given. Didn't find a process for decision−making or overcoming barriers.

Team Composition:

Does the lead principal investigator have successful management history and experience leading collaborative teams? Is it clear that all key personnel are committed to making significant contributions to the project? Do team members have complementary skills?

adequate
PI seems to have successful history, team has complementary skills and are committed to the project.

Communication Of Results:

Is there a clear plan for comprehensive and cohesive reporting of project progress to the CALFED community?

adequate
Dissemination includes: quarterly reports to CALFED, final technical report, presentations at CALFED Science Conference, IEP and manuscripts submitted to peer−reviewed journals.

Additional Comments:

Collaboration Panel (Discussion) Review

Primary reviewer rated proposal as adequate. Secondary reviewer rated it superior. After some panel discussion of details discussed in the proposal, the secondary reviewer

conceded to the primary reviewer's strong background in the study area, and subsequent judgement.

Technical Synthesis Panel Review

Proposal Title

#0318: ARE ‘APPARENT’ SEX REVERSED CHINOOK SALMON A SYMPTOM OF GENOTOXICITY?

Final Panel Rating
above average

Technical Synthesis Panel (Primary) Review

TSP Primary Reviewer's Evaluation Summary And Rating:

The proposal should provide important insight into the mechanism responsible for the “apparent−XY−females” in fall−run Chinook salmon. The approach is generally solid (one issue could be the resolution of the in−situ hybridization method), but the investigators have a strong reputation and are experienced in this research, so it is very likely that they will successfully address the issue. However, the research does not address the proposal−title question (whether the phenomenon is due to genotoxicity – it may not be since it is not limited to the Central Valley; it may be a relatively old mutation), and the proposed research at best only indirectly addresses the magnitude of any negative effect is has at the population level. The research will produce useful basic science, which could be relevant to broader goals of CALFED.

Additional Comments:

EXTERNAL REVIEWS: External reviews were generally complimenatary of the proposed research, as well as the PIs past performance and likelihood of success here. One review noted that the applied aspects of the project may be fairly limited. The hypotheses and objectives were generally clear.

However, the stated objective that the phenomenon is due to genotoxicity, is not addressed in the proposal. The project was properly justified, and the proposed research was considered to be a justified extension of previous research on this issue. The approach was considered to be very appropriate for addressing the chromosoal changes responsible for the "apparent XY females" (though not for addressing the genotoxicity issue). One potential problem could be that the lack of resolution of the FISH assay may be insufficient for distinguishing between the two mechanisms. The project was considered to be technically feasible. The product of the project (distinguishing between potential underlying mechanisms for the phenomenon) would be a valuable product. Important future research would be to determine if the XY females have a reduced fitness relative to control (XX) females. The budget was considered to be reasonable and adequate, though it was not clear why Dr. May would be receiving compensation for the cytogenetic work (to be done by Dr. Philips).

The proposal should provide important insight into the mechanism responsible for the "apparent-XY-females" in fall-run Chinook salmon. The approach is generally solid (one issue could be the resolution of the in-situ hybridization method), but the investigators have a strong reputation and are experienced in this research, so it is very likely that they will successfully address the issue. However, the research does not address the proposal-title question (whether the phenomenon is due to genotoxicity – it may not be since it is not limited to the Central Valley; it may be a relatively old mutation), and the proposed research at best only indirectly addresses the magnitude of any negative effect is has at the population level. The research will produce useful basic science, which could be relevant to broader goals of CALFED.

Technical Synthesis Panel (Discussion) Review

TSP Observations, Findings And Recommendations:

Are ‘apparent’ sex reversed Chinook salmon a symptom of genotoxicity

The work proposed in this study is well designed, presented and cost effective. It addresses an important question of scientific merit regarding identification of the mechanism for apparent sex reversal in Chinook salmon. The panel recognized the potential implications of this study with regard to population level responses, although these issues were not developed in the proposal. In addition, the link with genotoxicity (reflected in the title of the proposal) is not addressed in the actual project.

Final Ranking: Above Average

Technical Review #1

proposal title: ARE ‘APPARENT’ SEX REVERSED CHINOOK SALMON A SYMPTOM OF GENOTOXICITY?

Review Form

Goals

Are the goals, objectives and hypotheses clearly stated and internally consistent? Is the idea timely and important?

Comments	**The projects goals &objectives are clearly articulated and consistent with topics and goals stated for CALFED (especially species at risk and ecosystem restoration). The hypotheses are presented upfront to address three significant questions (based on information needs).** **The central idea behind the project will link critically important biological observations (sex reversal and feminization in salmon) with genetic and environmental causes (mutation and genotoxicity). As such this is a highly fundable project in terms of relevancy, design, and approach.**
Rating	**very good**

Justification

Is the study justified relative to existing knowledge? Is a conceptual model clearly stated in the proposal and does it explain the underlying basis for the proposed work? Is the selection of research, pilot or demonstration project, or a full−scale implementation project justified?

Comments	**The project takes advantage of the foundational work completed and reported by two of the investigators. The current proposal is a reasonable and logical**

	extension and follow-through of this earlier work. **It should complement current theoretical constructs and information for sex change mechanisms not only in salmon, but other vertebrates as well.** **As the investigators have previously demonstrated that the methodologies and approaches are workable, full funding is warrented.**
Rating	**very good**

Approach

Is the approach well designed and appropriate for meeting the objectives of the project? Is the approach feasible? Are results likely to add to the base of knowledge? Is the project likely to generate novel information, methodology, or approaches? Will the information ultimately be useful to decision makers?

Comments	**The approach is appropriate to meeting the project's objectives. The project will significantly add to our understanding of some of the subtle complexities of biological life cycles in salmon.** **A primary benefit to resource decision-makers will be a critical understanding of the role contaminants (such as endocrine disrupters) may play in the decline in salmon (Sac-SJ system and more broadly).** **The approach ultimately benefits from controlled breeding combined with powerful molecular and cytogenetic methods in the lab.**
Rating	**excellent**

Feasibility

Is the approach fully documented and technically feasible? What is the likelihood of success? Is the scale of the project consistent with the objectives and within the grasp of authors?

Comments	**The approaches are apparently routinely used by the investigators indicating that work is doable and will be completed in a timely manner without unforeseen complications.**
Rating	**excellent**

Monitoring

If applicable, is monitoring appropriately designed (pre−post comparisons; treatment−control comparisons)? Are there plans to interpret monitoring data or otherwise develop information?

Comments	**The project is research based and is not ammenable to "monitoring" other than meeting timelines and milestones. The projects duration is sufficiently compact that delivery of products and outputs will be observable by the funding agency.**
Rating	**very good**

Products

Are products of value likely from the project? Are contributions to larger data management systems relevant and considered? Are interpretive (or interpretable) outcomes likely from the project?

Comments	**The project addresses a key uncertainty in our understanding of sex determination and the relative contributions of genetics and the environment.** **As such, the key product will be a robust information set to assist managers and decision−makers. Moreover, the investigators' records indicate a consistent pattern of information and technology transfer to project sponsors and the scientific community at−large.**
Rating	**very good**

Additional Comments

Comments

Capabilities

What is the track record of authors in terms of past performance? Is the project team qualified to efficiently and effectively implement the proposed project? Do they have available the infrastructure and other aspects of support necessary to accomplish the project?

Comments	**The investigators have a significant record of past performance in creative research relevant to resource managers and decision-makers. Recent achievements also indicate that the proposed project is very doable and will yield clear answers to their questions. Assets and general support is available through the investigators' home institutions - the build-up of new infrastructure in not required to accomplish the project's goals.**
Rating	**excellent**

Budget

Is the budget reasonable and adequate for the work proposed?

Comments	**The budget is reasonable for the scope of the project and in-line with joint laboratory and fish rearing projects (in total and by task). In addition, the contribution of in-kind time by Williamson reflects a value-added efficiency to the project's completion.**
Rating	**very good**

Overall

Provide a brief explanation of your summary rating.

Comments	**Overall, this project will provide critical information linking genetics, environmental contaminants, and the history and perhaps fate of**

	salmon. The project is well-designed to address its objectives and hypotheses, addresses vital information needs identified by CALFED, budget-appropriate, and feasible given the institutions and records of achievement by the investigators.
Rating	**very good**

Technical Review #2

proposal title: ARE ‘APPARENT’ SEX REVERSED CHINOOK SALMON A SYMPTOM OF GENOTOXICITY?

Review Form

Goals

Are the goals, objectives and hypotheses clearly stated and internally consistent? Is the idea timely and important?

Comments	**The primary goal of this proposal is to test three hypotheses that will aid in the CALFED Ecosystem Restoration objective for fall−run Chinook with respect to chemical stressors on population viability. Each hypothesis is clearly stated, internally consistent, and easily attainable in the time frame of this proposal. This work is extremely interesting to the genetics and environmental toxicology communities by providing an important step forward for elucidating the underpinnings of sex reversal in Chinook salmon and potential correlates among environmental stressors and sex−reversal.**
Rating	**very good**

Justification

Is the study justified relative to existing knowledge? Is a conceptual model clearly stated in the proposal and does it explain the underlying basis for the proposed work? Is the selection of research, pilot or demonstration project, or a full−scale implementation project justified?

Comments	**The present work builds upon knowledge gleaned from several existing studies that show sex−reversal (in the study organism) is due to a heritable mutation rather than altered differentiation during development. Furthermore, the markers used in this study have been well−characterize in previous studies.**

	Thus, this study in one of several logical steps to further identify and understand the mechanism involved in sex−reversal of Chinook salmon. The model is clearly explained and stated null hypotheses are testable using the model proposed by the authors. I am concerned however, that the primarly goal is to relate data gleaned from this project to the relative importance of chemical stressors on population viability. After talking at length about the potential problems with genotoxic compounds and population dynamics, the authors note "the events that produce apparent sex−reversed individuals do not appear to be temporally or geographically limited to California's Central Valley." Are they suggesting that genotoxic compounds are found in many river systems (they only cite a manuscript in submission)? Sex reversal has also been document in the Columbia River (Chowen and Nagle 2004). First, to clearly obtain the goal of this proposal, there needs to be a correlation between sex reversed individuals and genotoxic compounds – why not measure water quality in these same watersheds? Second, the idea that this mutation is not temporally or geographically limited suggests that it may be an relatively ancient mutation held at low frequency under balancing selection (frequency dependent selection), and may not be correlated at all with genotoxic compounds.
Rating	**good**

Approach

Is the approach well designed and appropriate for meeting the objectives of the project? Is the approach feasible? Are results likely to add to the base of knowledge? Is the project likely to generate novel information, methodology, or approaches? Will the information ultimately be useful to decision makers?

Comments	**As stated above, the approach is not designed to address the overlying goal of the relative importance of chemical stressors on population viability. Having said this, the approach is**

	very well designed and appropriate from discriminating between the various hypotheses pertaining to the elucidation of the chromosomal mechanism responsible for producing the apparent XY− female phenotype, as outlined in the proposal. The underlying mechanism generating this XY−female phenotype is of great interest to evolutionary biologists studying sex determination in diploid organism. Probably of more interest, as stated by the authors, is the potential impacts that competing mechanisms hold for the Chinook salmon populations – something that should have been expanded upon further in this proposal.
Rating	very good

Feasibility

Is the approach fully documented and technically feasible? What is the likelihood of success? Is the scale of the project consistent with the objectives and within the grasp of authors?

Comments	The document is technically feasible and has a very high likelihood of success. The methodology is straight forward, and the scale is consistent with the objectives in the time frame allotted.
Rating	excellent

Monitoring

If applicable, is monitoring appropriately designed (pre−post comparisons; treatment−control comparisons)? Are there plans to interpret monitoring data or otherwise develop information?

Comments	N/A
Rating	not applicable

Products

Are products of value likely from the project? Are contributions to larger data management systems relevant and considered? Are interpretive (or interpretable) outcomes likely from the project?

Comments	**Products of value are very likely to come from this project. It is a well-designed and technically feasible project headed by leading experts in their fields. In one sense, data from this project will not contribute extensively to larger data management systems, but the data from this project will lay down a foundation to build upon for subsequent studies, especially if the mechanism has long-term implication for genetic diversity in Chinook salmon. Lastly, the project, as designed, will have interpretive results for the underlying mechanism leading to the XY-female Chinook phenotype.**
Rating	**very good**

Additional Comments

Comments

Capabilities

What is the track record of authors in terms of past performance? Is the project team qualified to efficiently and effectively implement the proposed project? Do they have available the infrastructure and other aspects of support necessary to accomplish the project?

Comments	**This is a highly qualified team of researchers both in population genetics and quantitative genetics. Their track record for publication and research indicates that they can efficiently and effectively implement the proposed work. Between the PIs, the available infrastructure and support is available to accomplish this project in a timely manor.**
Rating	**excellent**

Budget

Is the budget reasonable and adequate for the work proposed?

Comments	**A very reasonably proposed budget**
Rating	**excellent**

Overall

Provide a brief explanation of your summary rating.

Comments	**This a very coherent study that will address the underlying mechanism for sex reversal in Chinook salmon, and although it is more a molecular study than a conservation or management one, the potential significance and impact of long-term viability could be great if a causal link between genotoxicity and the XY-female mutation is found. Unfortunately the proposal falls short because this link is not discussed in this proposal, leaving me wondering if this work (although very interesting) is of high priority.**
Rating	**good**

Technical Review #3

proposal title: ARE ‘APPARENT’ SEX REVERSED CHINOOK SALMON A SYMPTOM OF GENOTOXICITY?

Review Form

Goals

Are the goals, objectives and hypotheses clearly stated and internally consistent? Is the idea timely and important?

Comments	**The hypotheses are clearly stated and the proposed project will address all three hypotheses. The first stated objective in testing these hypotheses is to "remove uncertainty regarding whether or not XY−female fish negatively impact populations genetics and persistence [6]." This important objective is likely to be achieved through the proposed research. However, the other stated objective is to determine whether sex chromosome alterations "are a symptom of genotoxicity experienced by fall populations due to exposure to environmental contaminants [6]," and it is not clear from the proposal how this question will be addressed. Both the alternative outcomes (i.e., a translocation of Y alleles onto the X, or a loss of function of sex determining alleles on the Y) could be the result of environmental toxicity, or neither may be. The proposed research does not include a test this hypothesis. Nonetheless, in my view the first stated objective and the methods proposed to address it are worthy of funding.**
Rating	**excellent**

Justification

Is the study justified relative to existing knowledge? Is a conceptual model clearly stated in the proposal and does it explain the underlying basis for the proposed work? Is the selection of research, pilot or demonstration project, or a full−scale implementation project justified?

Comments	**The authors appear to have a detailed understanding of the system in which they are working, and the proposed research is clearly the next step.**
Rating	**excellent**

Approach

Is the approach well designed and appropriate for meeting the objectives of the project? Is the approach feasible? Are results likely to add to the base of knowledge? Is the project likely to generate novel information, methodology, or approaches? Will the information ultimately be useful to decision makers?

Comments	**The challenge will be to obtain sufficient resolution in the FISH assay to distinguish among the stated alternatives, particularly as the proposal does not make clear to what extent the staining patterns of normal crosses are currently known or resolved. R. Phillips has a strong track record in cytogenetic analysis, however, so if any lab is going to achieve this objective, this one will. The clarity of the predicted alternative outcomes is also appealing.**
Rating	**very good**

Feasibility

Is the approach fully documented and technically feasible? What is the likelihood of success? Is the scale of the project consistent with the objectives and within the grasp of authors?

Comments	**See above with regard to the challenge of obtaining sufficient resolution with the FISH assay. The scale of the project is consistent with the objectives of the proposal and is well within the realm of the authors' expertise.**
Rating	**very good**

Monitoring

If applicable, is monitoring appropriately designed (pre−post comparisons; treatment−control comparisons)? Are there plans to interpret monitoring data or otherwise develop information?

Comments	**n/a**
Rating	**not applicable**

Products

Are products of value likely from the project? Are contributions to larger data management systems relevant and considered? Are interpretive (or interpretable) outcomes likely from the project?

Comments	**The products of the research, namely determining whether XY−females contain a compromised Y chromosome or an altered, but functional, X chromosome has direct bearing on the effect of XY−females at the population level. If the latter proves true, further research might entail estimating fitness of XY−females relative to control females.**
Rating	**excellent**

Additional Comments

Comments	

Capabilities

What is the track record of authors in terms of past performance? Is the project team qualified to efficiently and effectively implement the proposed project? Do they have available the infrastructure and other aspects of support necessary to accomplish the project?

Comments	**Authors appear uniquely qualified to conduct this research.**
Rating	**excellent**

Budget

Is the budget reasonable and adequate for the work proposed?

Comments	**It is not clear to my why Dr. May is receiving compensation for the cytogenetic work (Task 3) if this work is being conducted by Dr. Phillips [p. 8, p. 13]. Otherwise the budget seems reasonable and appropriate.**
Rating	**very good**

Overall

Provide a brief explanation of your summary rating.

Comments	**Although the proposed research does not directly address the question that forms the title of the application (are XY−females the result of genotoxicity), nonetheless the information obtained is critical to our understanding of this phenomenon and has important implications at the population level.**
Rating	**excellent**